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Short communication

Expanding the canvas of *PRKN* mutations in familial and early-onset Parkinson disease

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ABSTRACT

Background: Mutations in *PRKN* (PARK2) are commonly encountered in early-onset Parkinson disease (PD).
Objectives: To screen for *PRKN* mutations in a clinically well-characterized cohort of early-onset PD patients with a family history (FEOPD; ≤50 years at onset) or sporadic (SEOPD; ≤50 years at onset) and late-onset familial patients (FLOPD; > 50 years at onset).
Methods: A total of 97 patients including 52 SEOPD and 45 familial PD (FEOPD: 23; FLOPD: 22) were screened for variants in *PRKN* by PCR- Sanger sequencing. *PRKN* dosage and variants in known PD genes were screened by qPCR and whole-exome sequencing in a subset of samples.
Results: A total of 25 (25.77%) patients (SEOPD: 12, FEOPD: 6, and FLOPD: 7) were positive for *PRKN* variants. Of these, two patients manifested homozygous variants; while one patient was carrying three *PRKN* variants and two patients were carrying two *PRKN* variants. But, we could not examine their parents or relatives and their genotypes remain unknown. The remaining 20 (80%) patients were carrying heterozygous variants only. 32% of these variants were in exon 2, including a novel truncating homozygous variant (c.97C > T:p.Arg33Ter) in a SEOPD patient.
Conclusion: In our cohort, a novel homozygous variant (c.97C > T:p.Arg33Ter) in a patient with hyperhidrosis expands the spectrum of *PRKN* associated mutations. Furthermore, ~80% of the *PRKN* variants being heterozygous in this study cohort, implies the utility of the cohort for identification of additional novel/known causative PD gene(s).

1. Introduction

Yamamura and colleagues first reported 15 cases of autosomal recessive juvenile Parkinson disease (PD) in 1973 [1]. Twenty-five years later in 1998, Kitada and colleagues reported mutations in *PRKN* causing autosomal recessive PD (ARJP), which was followed by several more reports from across populations [2,3]. Published studies have shown an increased likelihood of pathogenic mutations in patients with an earlier age of onset [4]. Mutations in *PRKN* are a major contributor to juvenile and early-onset PD [4,5]. In a systematic review by Kilarski et al., pathogenic *PRKN* mutations were found in 8.6% of early-onset PD cases [5]. Some important clinical features such as dystonia, dyskinesia, and motor fluctuations have been described in patients harboring *PRKN* mutations [6]. However, due to an overall limited availability of phenotypic data from *PRKN* mutation-positive patients as detailed in a recent systematic review, which, included 958 *PRKN* mutation carriers originated from 663 families [7], it has not been possible to establish definitive genotype-phenotype correlations, demanding further investigations in well phenotyped patient cohorts [7]. In this study, we attempted to identify *PRKN* variants in one such cohort from north India comprising early-onset PD patients with a family history (FEOPD; ≤50 years at onset) or sporadic PD patients (SEOPD; ≤50 years at onset) and late-onset familial PD patients (FLOPD; > 50 years at onset).

2. Methods

PD patients were recruited at GIPMER, New Delhi, (a tertiary care

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teaching institute) after approval from the institutional ethics committee (IEC). Written informed consent was obtained from all participating individuals as per the IEC guidelines. Familial Parkinson disease (FPD) patients were further classified as early-onset (FEOPD; ≤50 years) and late-onset (FLOPD; > 50 years). A total of 97 patients, including 45 FPD (FEOPD: 23; FLOPD: 22) and 52 SEOPD patients were evaluated and their detailed history and clinical information were recorded with the help of a pre-designed form. Diagnosis of PD was made based on "UK Parkinson disease society brain bank clinical diagnostic criteria". Movement disorder society-Unified Parkinson disease rating scale (MDS-UPDRS) was used during the 'on' and 'off' state for the clinical evaluation of both motor and non-motor features. Mini-mental scale evaluation (MMSE) was used for memory and cognitive assessment. About 5 ml of peripheral blood samples were collected in EDTA vacutainers from all the subjects recruited in the study.

2.1. Genetic analysis

DNA was isolated using a standard phenol-chloroform extraction protocol routinely used in the genetics laboratory. DNA was subject to quantitative and qualitative analysis and used for subsequent genetic analyses. Screening for variants in the complete coding regions of *PRKN* encompassing 12 exons, was done by PCR amplification followed by Sanger sequencing, using primers previously reported [8]. Sanger sequencing with the reverse primer confirmed variants identified and phenotype-genotype correlations, if any, were established. PD subjects with homozygous or carrying two or three *PRKN* variants were whole-exome sequenced (WES) to assess copy number variations in *PRKN* and contribution of other known PD genes if any. Furthermore, the presence/absence of gene rearrangements in these cases was validated by qPCR in deletion hotspots including exons 2, 3, 5 and 10.

2.2. Statistical analysis

Clinical data were analyzed using the "Statistical Package for the Social Sciences (SPSS)" PC-23 version. Data were presented as mean, standard deviation (SD) of the mean, and range for age at presentation, age at disease onset, disease duration, duration of levodopa treatment, MMSE, MDS-UPDRS part III and Hoehn and Yahr (H & Y) stage. 'Fisher's exact test' was used to compare variables between SEOPD and LOPD groups.

3. Results

Clinical: As for the clinical details of the study cohort (Table 1), the majority (7/12; 58.33%) of the cases in the SEOPD group were akinetic-rigid type. Scores of MDS-UPDRS and H&Y were not significantly different between the groups. Levodopa-induced dyskinesia was seen in only the EOPD group. The mean MMSE was low in our patient cohort, and many of our patients lacked formal education.

Genetic analysis: A total of 97 patients (52 SEOPD and 45 FPD (FEOPD: 23; FLOPD: 22) were screened for mutations in all the 12 exons of *PRKN* by PCR- Sanger sequencing. Twenty-five (25.77%) patients (SEOPD: 12, FEOPD: 6, and FLOPD: 7) harbored *PRKN* variants, which included one non-sense, 21 non-synonymous and three synonymous variants. Of these, two patients manifested homozygous variants; while one possessed three *PRKN* variants and two patients had two *PRKN* variants (Supplementary online Table 1). The remaining 20 (80%) patients manifested heterozygous variants only (Supplementary online table 2). Of note, 32% of the total observed variants were in exon 2, including a truncating variant **(**c.97C > T:p.Arg33Ter**)** observed in a homozygous state for the first time in a SEOPD patient. Remaining variants were distributed across different exons (Supplementary online Tables 1 and 2).

3.1. Case with homozygous variants

Case 1 (Video 1): A 19-year-old male presented with excessive sweating (hyperhidrosis) in his hands and soles for one year and tremor for three months. There was no family history of tremor or PD. On examination, he had tremors in bilateral upper and lower limbs (left > right), face, and tongue. His magnetic resonance imaging (MRI) of the brain and blood tests (including thyroid and parathyroid profiles) were normal. He was treated with tablets of propranolol (40 mg two times daily) and trihexyphenidyl (2 mg three times daily) for 2 months, but there was no improvement in hyperhidrosis and tremor. He was injected with Onabotulinum toxin in the palms and soles (total 400 U). At three months follow-up, hyperhidrosis had significantly improved, but he had developed slowness and leaning on the right side. His tremors had also worsened during this period. On examination, he had bilateral rest and action tremor in upper and lower limbs (left > right), bradykinesia (left > right) and rigidity in neck, upper and lower limbs (left > right). Considering a diagnosis of PD, a dopamine transporter (DaT) scan was done, which showed asymmetric bilateral loss of putamen DaT binding.

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PRKN variant analysis identified a homozygous truncating variant p.Arg33Ter in exon 2. Further, WES analysis ruled out the contribution of any other known PD genes, making this variant as the most likely

Table 1
Comparison of demographic and clinical features of Parkinson disease patients with *PRKN* mutations.

Demographic and clinical features	SEOPD (n = 12)	FEOPD (n = 6)	FLOPD (n = 7)
Male: Female	8:4	4:2	3:4
Age at onset in years (Mean ± SD; range)	33.33 ± 10.35; 12-48	35.33 ± 13.70, 11-48	59.42 ± 7.18, 51-70
Duration of disease in years (Mean ± SD; range)	6.33 ± 5.49; 1-14	8.33 ± 6.37, 1-15	1.42 ± 0.53, 1-2
Tremor dominant: Akinetic rigid type	5:7	4:2	3:4
MMSE score (Mean ± SD; range)	26.58 ± 3.65; 21-30	24.83 ± 4.44; 17-30	24.28 ± 7.45; 13-30
MDS-UPDRS I (Mean ± SD; range)	5.33 ± 7.31; 0-26	9.66 ± 4.96; 4-18	6.28 ± 5.58; 2-17
MDS-UPDRS II (Mean ± SD; range)	9.25 ± 9.41; 1-36	10.66 ± 7.52; 0-22	4.85 ± 3.43; 1-12
MDS-UPDRS III (OFF) (Mean ± SD; range)	35 ± 21.65; 3-84	33.33 ± 21.63; 8-61	22.85 ± 16.62; 6-54
H&Y scale (Mean ± SD; range)	2.66 ± 0.77; 1-4	2.50 ± 0.83; 1-3	1.85 ± 0.89; 1-3
Levodopa equivalent doses (LEDD)	572 ± 150.18; 375-800	466.66 ± 180.04; 200-650	485.71 ± 101.91; 375-650
Levodopa induced dyskinesia	5	0	0

SEOPD: Sporadic early onset Parkinson disease.
FEOPD: Familial early onset Parkinson disease.
FLOPD: Familial late onset Parkinson disease.
MMSE: Mini Mental State Examination.
MDS-UPDRS: Movement disorder society-Unified Parkinson Disease Rating Scale.
H&Y: Hoehn and Yahr; SD: Standard deviation.

cause of disease phenotype in this individual. The patient was treated with levodopa + carbidopa (100 + 25 mg four times daily). At one-year follow-up, his symptoms of hyperhidrosis, tremor, and slowness (MDS-UPDRS part III off score decreased from 18 to 8) had improved.

4. Discussion

Screening for mutations in a large number of PARK genes in well-characterized PD cohorts across age groups and ethnicities over the last two decades have unraveled the underlying genetics but of only a small proportion of PD cases. A wide phenotypic spectrum has been reported among these mutation-positive patients, reiterating extensive clinical heterogeneity. Taken together, effective genotype-phenotype correlations remain a challenge and thus continuous documentation of the mutation burden along with detailed phenotypic assessment in *trans*-ethnic settings may be a valuable contribution. 25% of the patients in this study showed variants in *PRKN,* wherein only 20% of these variant positive patients were homozygous or carrying two/three *PRKN* variants (Supplementary online Table 1). As mentioned in the results section, 80% of the variants in the study cohort were heterozygous (Supplementary online table 2) with their frequency ranging from 0.07 to 8×10^{-6} in Exac global and 0 to 0.08 in an ethnicity matched laboratory database. This excess heterozygous mutation profile implies that mutations in *PRKN* may not be the only genetic determinants of PD in these individuals, but one or more genes (novel/known), hitherto unidentified in this study may also contribute to their PD phenotype. For assessing the contribution of other known PD genes, if any, in five PD subjects with homozygous or carrying two/three *PRKN* variants (Supplementary online Table 1), we performed WES but no gene variant in any known PD genes were observed in individuals with homozygous *PRKN* variants. Interestingly, all three patients with carrying two/three *PRKN* variants had single base substitutions in *VPS13C*, suggesting a digenic contribution in them. On the other hand, this is the first report where a truncating mutation p.Arg33Ter has been observed in a homozygous condition in a SEOPD patient (Supplementary online Table 1). This patient had hyperhidrosis before the onset of motor symptoms. Hyperhidrosis in PD before the onset of motor symptoms has been rarely reported [9]. Of note, this phenotype has not been described in a recent systematic review [7]. This review also highlighted the lack of data for both cardinal (7–78%) and non-cardinal (73–97%) symptoms and therefore, the imminent need for systematic reporting of phenotypes particularly given the increasingly available molecular genetic testing [7].

The major limitation of our study has been the sole focus on *PRKN* variants despite the well-documented genetic heterogeneity in PD. This was essentially driven by the very high frequency reported in *PRKN* in EOPD. We would, however, be cautious in interpreting the *PRKN* mutation frequency data based on the findings in our study since sample recruitment included early and familial onset PD patients only, which may not be truly representing the frequency of *PRKN* mutations in the general PD population (Supplementary online table 2) and dosage variations which constitute significant proportion of *PRKN* mutations were not assessed in all study samples. Yet another deviant observation was the comparatively low MMSE scores in our patient cohort, which, is contrary to the reported literature.[7] But, our data is limited by the fact that many of our patients lacked formal education and we have not done detailed cognitive studies of these patients. Also, in some patients, there might have been other disorders among those not examined by WES.

In conclusion, our study identified *PRKN* variants with early-onset and familial PD, including a novel homozygous variant (p.Arg33Ter) in exon 2, in a SEOPD subject. Furthermore, ~80% of the *PRKN* variants being heterozygous in this study cohort, implies the utility of the cohort for identification of additional novel/known risk conferring PD gene(s).

Financial disclosure/conflict of interest

None.

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Roles of the authors

Sanjay Pandey and Laxmikant Ramkumarsingh Tomar were responsible for the diagnosis, recruitment, clinical characterization, interpretation of clinical data and blood collection for all study subjects. Sumeet Kumar carried out genetic analysis including Sanger sequencing and whole-exome sequencing data analysis and compilation. Shreya Dinesh performed qPCR for a subset of samples. SK, SD & BKT analyzed and interpreted the genetic data. SP, LRT, SK, and BKT wrote the first draft of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.08.005.

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